SUOMALAISEN TIEDEAKATEMIAN TOIMITUKSIA
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II. CHEMICA

104

# NUTRITIONAL REQUIREMENTS OF LACTIC ACID BACTERIA

## I. THE CALCIUM REQUIREMENTS OF *STREPTOCOCCUS THERMOPHILUS* STRAINS

HELSINKI 1961
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## Nutritional requirements of lactic acid bacteria

From the standpoint of growth factor requirements lactic acid bacteria are among the most complex of the micro-organisms that have been investigated. The growth factors which either stimulate or are necessary for growth of lactic acid bacteria include various amino acids, peptides, vitamins of the B complex (and related compounds), purine and pyrimidine bases and their derivatives, and fatty acids. The known growth factor requirements of these bacteria have been discussed in numerous reviews [1–7]. Certain strains of lactic acid bacteria, including those which are commonly used in the dairy industry (*e.g.* the strains *Streptococcus thermophilus, Lactobacillus helveticus*, *Lactobacillus lactis*, and *Lactobacillus bulgaricus*) require unknown factors for growth in synthetic media. A more complete knowledge of the nutritional requirements of the lactic acid bacteria used in cheese manufacture is therefore a necessary foundation for any improvements in the quality of cheese.

Advantage can be taken of the fact that lactic acid bacteria require unknown organic growth factors to detect, isolate and identify such factors in biological materials. Until now, however, this possibility of discovering new growth factors by means of lactic acid bacteria used in cheesemaking has been very little utilized. The first aim of this work was to find basal media in which the growth of the lactic acid bacteria would be optimal and which could be used in attempts to isolate new growth factors. It was necessary, first of all, to find out in what way the different chemical compositions of the growth media affect the growth of lactic acid bacteria.

## Experimental

### *Cultures and their maintenance*

The experiments were carried out on 124 strains of lactic acid bacteria which had been isolated as pure cultures from various Finnish dairy products (milk, butter, buttermilk, cheese). The strains were kindly supplied by

Professor A. I. Virtanen, Director of the Biochemical Institute, Helsinki. These lactic acid bacteria represent a large group of bacteria whose nutritional requirements have not yet been investigated. Some of the strains are commonly used in the manufacture of cheese in Finland.

Cultures of the organisms were maintained as stab cultures in test tubes containing agar medium (TSHGA- agar medium) or sterile skim milk. The TSHGA- agar medium contained 1 per cent glucose, 0.5 per cent lactose, 0.5 per cent yeast extract, 0.5 per cent sucrose, 0.5 per cent Bacto-tryptone, gelatine 0.25 per cent, ascordic acid 0.07 per cent, and 1.5 per cent agar. The pH was adjusted to 6.7—6.8. The frequency of transfer varied from once a fortnight to once a month. The cultures were maintained at 4° between transfers.

## *Preparation of inoculum cultures*

The composition of the inoculum medium was that described above but without the agar. This medium was divided into 7-ml aliquots which were sterilized in an autoclave 10—15 min. at 112° and then kept in a refrigerator. The inocula were prepared by transferring the organism from the stab culture to this medium. After being incubated at 37° for 12—48 hrs, depending on the strain in question, the cells were centrifuged and washed with 5—10 ml of 0.9 per cent sodium chloride solution. The centrifugation and washing were repeated three times. Finally the cells were suspended in 50 ml of saline. One drop of the slightly opaque suspension was used to inoculate the medium in each tube. Growth experiments were carried out at 37 or 42°. In general, the incubation was stopped when the logarithmic growth phase was reached in order to make possible a comparison of the rates of growth of different strains.

## *Compositions of basal media*

The compositions of the basal media used in the investigation are shown in Table 1. All the media are modifications of the growth medium for lactic acid bacteria described by Anderson and Elliker [8]. They were prepared by mixing stock solutions of the various compounds. Separate solutions of vitamins, purine and pyrimidine bases were prepared, but the inorganic salts were combined in a single solution unless stated otherwise. The basal media were adjusted to pH 6.7 before being used. All stock solutions were stored in a refrigerator and replaced monthly.

**Table 1.**

The compositions of basal media (quantities per 100 ml).

| Component | Quantity | S | SII | SIII | SIV | BI | BII | CI | CII | CIII | CIV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose | mg | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Lactose | » | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| Sucrose | » | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| DL-Aspartic acid | » | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| L-Glutamic acid | » | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Sodium acetate | » | 2000 | 2000 | 2000 | 2000 | — | 1000 | — | 1000 | 34 | 340 |
| Xanthine | » | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Casamino acids | » | 1000 | 1000 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| Tween 80 | » | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Oleic acid | » | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| DL-Tryptophan | » | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| L-Cystine | » | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Thymine | µg | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Cytosine | » | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Hypoxanthine | » | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Adenine, Guanine, and Uracil, each | mg | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Ascorbic acid | » | 40 | 40 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Thiamine HCl | µg | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Riboflavin | » | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Nicotinic acid | » | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| P-Aminobenzoic acid | µg | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Ca-pantothenate | » | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Biotin | mµg | 100 | 100 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Vitamin $B_{12}$ | » | 22 | 22 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| Folic acid | » | 1000 | 1000 | 500 | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| Pyridoxal phosphate | » | 400 | 400 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Pyridoxaminephosph. | µg | 10 | 10 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Pyridoxamine HCl | » | 10 | 10 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Pyridoxal | » | 20 | 20 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Pyridoxine HCl | mg | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| $KH_2PO_4$ | » | 1000 | 1000 | — | — | — | — | — | — | 12 | — |
| $FeSO_4 \cdot 7\,H_2O$ | µg | 4000 | 4000 | 1330 | 400 | 400 | 400 | 400 | 400 | 400 | 400 |
| $MnSO_4 \cdot 4\,H_2O$ | mg | 13 | 13 | 3 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| $MgSO_4 \cdot 7\,H_2O$ | » | 80 | 80 | 40 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| KCl | » | — | — | 550 | 140 | 140 | 140 | 140 | 140 | 140 | 140 |
| NaCl | » | 4 | 4 | — | — | — | — | 4 | 4 | 4 | 4 |
| Glycero-2-phosphate | » | — | — | 78 | — | 156 | 156 | — | — | 468 | 468 |
| Sodium citrate . $2\,H_2O$ | » | 500 | — | — | — | — | — | — | — | — | — |
| Succinic acid | » | — | — | — | — | — | — | — | — | 295 | 295 |

Medium SIIIb is the same as SIV but contains also 156 mg of glycero-2-phosphate per 100 ml.

All media contained traces of ammonia salt because xanthine was dissolved in strong ammonia.

Sodium hydroxide or hydrochloric acid was used to neutralize the media to pH 6.8 and therefore the concentrations of $Na^+$ or $Cl^-$ ions in the media are somewhat higher than the table indicates.

*General procedure*

In principle the experimental procedure used in the study was the same as that used in this laboratory for the determination of vitamins and amino acids. Two and a half millilitres of the basal medium was added to a test tube and diluted with water or supplements to 5 ml. The test tubes were then plugged with cotton wool and autoclaved 5—10 min. at 112—115°. After being cooled, the tubes were inoculated and then incubated at 37 or 42°. The growth of the lactic acid bacteria was followed turbidimetrically, the extent of growth being recorded as galvanometer readings of a Klett-Summerson colorimeter with a 590—660 m$\mu$ filter. In the tables showing the results, the rates of growth are expressed directly as galvanometer readings.

## Results

In the initial stage of the work, several of the 124 strains of lactic acid bacteria tested appeared to possess very complex growth factor requirements. It also became clear during the first experiments that some strains of *Streptococcus thermophilus* require calcium for growth when synthetic media are used. Because it is very important to know the exact nutritional requirements of these calcium-requiring strains, the next step was a detailed investigation of the effect of different inorganic and organic components of the basal media on the growth of these bacteria. The results of these experiments are shown in Tables 2—25. It should be noted that these results relate to strains which are sensitive to calcium. About 30 strains (mainly *Streptococcus thermophilus*) of the 124 strains preliminarily tested were sensitive in this respect.

*Potassium.* The effect of potassium on certain strains is shown in Table 2.

**Table 2.**

Effect of potassium concentration.

| Basal medium | Strain | mg. of $K^+$/ml | | | | | | Incubation at 42°C, hours |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.7 | 1.4 | 2.1 | 2.9 | 4.3 | |
| SIII | Str 10 | — | 150 | 110 | 40 | — | — | 24 |
| » | Str 11 | — | 110 | 100 | 90 | 60 | — | 24 |
| » | Str 110 | — | 150 | 110 | 90 | — | — | 24 |
| » | Str 111 | — | 170 | 160 | 160 | 140 | 20 | 24 |
| » | Cas I | — | 150 | 170 | 130 | 60 | — | 70 |
| » | Cas J (7) | — | 200 | 230 | 325 | — | — | 70 |
| » | Kungsbölen k. | — | 190 | 190 | 190 | 180 | 150 | 24 |
| » | Kauhajoen k. | — | 190 | 170 | 120 | — | — | 24 |

As can be seen, potassium ions are required by all the strains. Already the lowest concentration of potassium (37 $\mu$moles per ml) effected maximal growth under the experimental conditions.

*Manganese.* The effect of manganese on the growth of 10 strains is seen in Table 3. The basal media used in these experiments were SII and SIII, but without the manganese sulphate. The results show that manganese ions are not generally required by these strains.

**Table 3.**

Effect of manganese concentration.

| Basal medium | Strain | $\mu$g $Mn^{++}$/ml | | | | | | | Incubation at 42°C, hours |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.0 | 1.0 | 3.0 | 5.0 | 7.5 | 12.5 | 25.0 | |
| SII | Str 10 | 125 | | | 130 | | 100 | 100 | 18 |
| » | Str 10 | 180 | | | 180 | | 170 | 170 | 40 |
| SIII | Str 10 | 75 | 77 | 82 | 88 | 91 | 90 | | 24 |
| » | Str 11 | 55 | 77 | 76 | 79 | 79 | 79 | | 24 |
| » | Str 110 | 155 | 160 | 148 | 146 | 142 | 140 | | 24 |
| » | Str 111 | 175 | 172 | 168 | 162 | 161 | 168 | | 24 |
| » | Kauhaj. k. | 179 | 174 | 172 | 160 | 178 | 165 | | 24 |
| » | Kungsb. k. | 210 | 201 | 198 | 195 | 200 | 191 | | 24 |
| » | Cas J (7) | 20 | 144 | 115 | 70 | | | | 24 |
| | Cas I | 146 | 170 | 146 | 165 | 57 | 29 | | 24 |
| SII | » » | 145 | | | 125 | 65 | 54 | 50 | 18 |
| » | » » | 175 | | | 215 | 75 | 25 | 33 | 24 |
| » | » » | 235 | | | 290 | 280 | 260 | 105 | 40 |
| » | Ylilied. k. | — | | | 115 | 125 | 98 | 108 | 18 |
| » | » » | — | | | 115 | 126 | 120 | 110 | 24 |
| » | » » | 166 | | | 183 | 185 | 166 | 179 | 40 |
| » | Lb helv. 1182 | 185 | | | 185 | 183 | 155 | 12 | 60 |

*Iron.* Table 4 shows the effect of iron on the growth of 9 strains. Iron (II) ions do not seem necessary for rapid growth under the experimental conditions used. With some strains, iron was observed to promote growth.

*Magnesium.* The magnesium requirements of 10 strains were tested. As can be seen from Table 5, magnesium ions are essential for the growth of most strains and stimulate the growth of the other strains.

*Citrate.* The preliminary experiments showed that sodium citrate inhibits the growth of certain strains of lactic acid bacteria (named Group II). These findings were confirmed and the experiments were extended to an

**Table 4.**

Effect of iron concentration.

| Basal medium | Strain | $\mu g$ Fe++/ml 0.0 | 0.8 | 1.6 | 3.2 | 4.0 | 4.8 | 8.0 | Incubation at 42°C, hours |
|---|---|---|---|---|---|---|---|---|---|
| SIII | Str 10 | 57 | 67 | 54 | 40 | | 35 | 26 | 24 |
| SII | Str 10 | 110 | 158 | 188 | 155 | 142 | | 90 | 24 |
| » | Str 10 | 140 | 165 | 198 | 150 | 160 | | 180 | 45 |
| SIII | Str 11 | 38 | 69 | 63 | 58 | | 50 | 74 | 24 |
| SII | Str 111 | 210 | 165 | 250 | 190 | 186 | | 180 | 45 |
| SIII | Str 111 | 155 | 148 | 157 | 145 | | 136 | 136 | 24 |
| » | Kauh. k. | 165 | 169 | 161 | 161 | | 144 | 122 | 24 |
| » | Kungsb. k. | 178 | 181 | 172 | 172 | | 150 | 150 | 24 |
| » | Str 110 | 138 | 132 | 119 | 113 | | — | — | 24 |
| » | Cas J (7) | 10 | 96 | 115 | 100 | | 117 | 95 | 24 |
| » | Cas I | 145 | 187 | 187 | 199 | | 170 | 132 | 24 |
| SII | » I | — | 97 | 135 | 105 | | 110 | 114 | 27 |
| » | » » | — | 115 | 115 | 97 | | 78 | 50 | 45 |
| » | Lb helv. 1182 | 20 | 78 | 129 | 206 | | 200 | 190 | 45 |

**Table 5.**

Effect of magnesium concentration.

| Basal medium | Strain | $\mu g$ Mg++/ml 0.0 | 20.0 | 40.0 | 60.0 | 80.0 | 120.0 | Incubation at 42°C, hours |
|---|---|---|---|---|---|---|---|---|
| SII | Str 10 | — | 125 | 125 | 120 | 107 | | 27 |
| SIII | Str 10 | — | 105 | 106 | 103 | 90 | 95 | 24 |
| SII | Str 10 | — | 153 | 174 | 178 | 178 | | 40 |
| SIII | Str 11 | 47 | 106 | 112 | 100 | 100 | 92 | 24 |
| » | Str 111 | — | 170 | 167 | 167 | 165 | 160 | 24 |
| » | Kauh. k. | — | 170 | 166 | 155 | 152 | 145 | 24 |
| » | Kungsb. k. | — | 194 | 195 | 198 | 206 | 209 | 24 |
| » | Cas J (7) | — | 115 | 230 | 226 | 210 | 140 | 72 |
| » | Str 110 | — | 128 | 111 | 138 | 46 | 11 | 24 |
| SII | Cas I | — | 15 | — | 10 | 55 | | 24 |
| » | » » | 30 | 230 | 220 | 270 | 185 | | 40 |
| SIII | » » | 12 | 185 | 192 | 189 | 174 | 173 | 72 |
| SII | Ylilied. k. | — | 130 | 135 | 120 | 110 | | 24 |
| » | » » | 167 | 184 | 185 | 166 | 179 | | 40 |
| » | Lb helv. 1182 | 5 | 24 | 16 | 14 | 47 | | 64 |

examination of the tolerance of citrate by 25 strains of Group II using basal medium SIII (or SII). The citrate tolerance varied considerably with different strains. The strains were divided into four groups according to the degree of tolerance (Table 6).

**Table 6.**

Tolerance of citrate by different strains of lactic acid bacteria.

| Group IIA, tolerates less than 1 mg | Group IIB, tolerates 1—2 mg | Group IIC, tolerates 2—3 mg | Group IID, tolerates more than 3 mg |
|---|---|---|---|
| Str 11 | Str 10 | Hatt.s. | Lb 80 |
| Str 40 | Str 11R | Lb lact 1183 | Lb 83 |
| Str 75 | Str 72 | B.c.h.H. | Lb helv 1182[1] |
| Str 100 | Str 74 | | |
| Str 102 | Str 101 | | |
| Str 103 | Str 111[1] | | |
| Str 110 | Cas I[2] | | |
| Ths[1] | Cas J (7) | | |
| Kauhaj.k. | H.th.str | | |
| Ylilied.k.[2] | | | |

[1]) Strain tested in medium SII.
[2]) Strain tested in media SII and SIII.

**Table 7.**

Effect of calcium.

| Strain | $\mu g$ $Ca^{++}$/ml | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | — | 1 | 2 | 3 | 4 | 5 | 10 | 15 | 20 | 30 |
| Str 10 | 5 | 18 | 6 | 2 | 95 | 128 | 125 | 135 | 130 | 130 |
| Str 10 | 3 | 14 | — | 6 | — | 115 | 135 | | 125 | 140 |
| Str 11 | 1 | 10 | 12 | 11 | 6 | 11 | 22 | 52 | 93 | 115 |
| Str 11 | 6 | 19 | 5 | 3 | 6 | 7 | 11 | 41 | 26 | 85 |
| Str 11R | 9 | 18 | 45 | 82 | 105 | 108 | 142 | 140 | 141 | 139 |
| Str 11R | 7 | 19 | 110 | 100 | 135 | 140 | 139 | 137 | 139 | 140 |
| Str 110 | — | 15 | 18 | 178 | 142 | 175 | 230 | 200 | 228 | 225 |
| Str 110 | — | 14 | 92 | 175 | 184 | 182 | 225 | 214 | 200 | 190 |
| Cas I | 39 | — | 39 | 63 | 94 | 117 | — | 195 | 240 | 244 |
| » » | 40 | 280 | 65 | 66 | 97 | 127 | — | 200 | 240 | 252 |

Basal medium S, in which the concentration of sodium citrate was 2.5 mg per ml, was used. Incubation 48 hrs at 42°.

*Calcium.* In a medium containing citrate, calcium is either necessary for or a stimulus to the growth of most strains. In a citrate-free basal medium, calcium has no effect on the growth in most cases (Table 8). However, as

**Table 8.**

Effect of calcium on growth in media containing citrate (SIVSS) and in citrate-free media (SIV). Incubation at 42°.

| Strain | SIVSS μg Ca++/ml | | | | Hours | SIV μg Ca++/ml | | | | Hours |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | | 0 | 5 | 10 | 15 | |
| Str 10 | — | — | 30 | 30 | e 24 | 50 | 50 | 45 | 45 | o 24 |
| Str 10R | — | — | — | — | 72 | — | 15 | 40 | 50 | e 24 |
| Str 11 | — | 35 | 45 | 45 | e 24 | 50 | 50 | 55 | 55 | o 24 |
| Str 11R | — | — | — | — | 72 | — | 50 | 40 | 40 | e 48 |
| Str 12 | — | — | 40 | 80 | e 72 | — | 15 | 45 | 60 | e 24 |
| Str 40 | — | 40 | 50 | 60 | e 48 | 60 | 60 | 60 | 65 | o 48 |
| Str 72 | — | — | 90 | 100 | e 24 | — | 80 | 100 | 105 | e 24 |
| Str 74 | — | 50 | 80 | 75 | e 48 | 70 | 70 | 70 | 75 | o 24 |
| Str 75 | — | 40 | 45 | 55 | e 48 | 70 | 60 | 60 | 70 | o 48 |
| Str 77 | — | — | 75 | 130 | e 72 | — | 30 | 60 | 80 | e 24 |
| Str 100 | — | — | 45 | 45 | e 48 | 55 | 60 | 65 | 60 | o 24 |
| Str 101 | — | — | 50 | 30 | e 48 | — | 5 | 20 | 20 | e 24 |
| Str 102 | — | 20 | 60 | 60 | e 48 | 35 | 45 | 45 | 50 | o 24 |
| Str 103 | — | — | 60 | 65 | e 48 | 40 | 55 | 60 | 55 | o 24 |
| Str 110 | — | 80 | 145 | 115 | e 24 | 155 | 120 | 130 | 135 | o 24 |
| Str 111 | | 80 | 120 | 105 | 24 | 140 | 125 | 120 | 105 | o 24 |
| Th | — | — | 45 | 80 | e 24 | 70 | 105 | 110 | 115 | s 24 |
| Ths | — | 5 | 30 | 105 | e 24 | — | 50 | 110 | 110 | e 24 |
| Cas I | — | 40 | 100 | 110 | e 24 | 110 | 125 | 125 | 120 | o 24 |
| Cas J (7) | 5 | 30 | 100 | 140 | s 24 | 130 | 140 | 140 | 140 | o 24 |
| H.th.str. | — | — | 10 | 5 | e 48 | — | 10 | 30 | 30 | e 24 |
| Hatt.s. | 20 | 55 | 75 | 75 | s 24 | 140 | 125 | 120 | 105 | o 24 |
| Kauh.k. | — | 130 | 150 | 160 | e 48 | 145 | 160 | 155 | 155 | o 48 |
| Kungsb.k. | 110 | 50 | 100 | 130 | 48 | 120 | 120 | 130 | 120 | o 48 |
| Lb 83 | 135 | 135 | 125 | 100 | o 48 | 150 | 130 | 125 | 130 | o 48 |
| Lb lact. 1183 | 10 | 130 | 125 | 105 | s 48 | 125 | 130 | 135 | 135 | s 48 |

Notes: e = calcium essential for growth.
s = calcium stimulates growth.
o = calcium has no effect on the growth.

can be seen from Tables 9 and 12, the composition of the basal media also otherwise affects the calcium requirements of the strains. On the basis of their calcium requirements in the SIV and SIVSS basal media, the strains were divided into three groups as shown in Table 10. These groups are:

**Table 9.**

Effect of calcium on the growth in media SII and SIIIb lacking citrate. Incubation at 42°.

| Strain | SIIIb µg Ca++/ml | | | | Hours | SII µg Ca++/ml | | | | | | Hours |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | | 0 | 5 | 10 | 15 | 20 | 25 | |
| Str 10 | 50 | 70 | 65 | 65 | o 24 | 125 | 115 | 110 | 105 | 105 | 105 | 0 24 |
| Str 10R | — | 5 | 30 | 45 | e 24 | | | | | | | |
| Str 11 | 45 | 60 | 60 | 60 | o 24 | 80 | 80 | 80 | 80 | 90 | 80 | o 24 |
| Str 11R | 45 | 55 | 55 | 60 | o 24 | 110 | 105 | 100 | 90 | 90 | 90 | o 24 |
| Str 12 | — | 5 | 20 | 35 | e 24 | | | | | | | |
| Str 40 | 70 | 70 | 70 | 70 | o 24 | 100 | 115 | 110 | 110 | 110 | 110 | o 24 |
| Str 72 | 25 | 120 | 130 | 130 | s 24 | — | 20 | 30 | | 100 | 100 | e 24 |
| Str 74 | 85 | 85 | 75 | 75 | o 24 | — | 120 | 120 | 120 | 120 | 125 | e 24 |
| Str 75 | 60 | 65 | 60 | 55 | o 24 | — | 20 | 100 | 80 | 70 | 110 | e 24 |
| Str 77 | — | 20 | 65 | 85 | e 24 | — | — | — | — | 5 | 15 | e 24 |
| Str 100 | 70 | 70 | 70 | 70 | o 24 | — | — | 5 | 20 | 25 | 20 | e 24 |
| Str 101 | 40 | 50 | 50 | | o 24 | — | 20 | 35 | 55 | 50 | 50 | e 24 |
| Str 102 | 25 | 50 | 55 | 60 | s 24 | — | — | 5 | 5 | 5 | 10 | e 24 |
| Str 103 | 45 | 70 | 70 | 65 | o 24 | — | 5 | 10 | 35 | 50 | 45 | e 24 |
| Str 110 | 160 | 120 | 120 | 125 | o 24 | 140 | 155 | 155 | 165 | 160 | 160 | o 24 |
| Str 111 | 145 | 130 | 135 | 135 | o 24 | 190 | 205 | 205 | 210 | 210 | 215 | o 24 |
| Th | 5 | 10 | 115 | 140 | s 24 | — | 15 | 100 | 135 | 150 | | e 24 |
| Ths | 150 | 160 | 175 | 190 | s 24 | — | 35 | 125 | 145 | 150 | 155 | e 24 |
| Cas I | 20 | 125 | 125 | 125 | s 24 | 185 | 290 | | 320 | | | s 24 |
| Cas J (7) | 170 | 180 | 200 | 195 | o 24 | — | 140 | 180 | 250 | 290 | 270 | e 24 |
| H.th.str | — | 45 | 60 | 60 | e 24 | — | — | 5 | 5 | 5 | 10 | e 24 |
| Hatt.s. | 180 | 180 | 190 | 180 | o 24 | | | | | | | |
| Kauh.k. | 125 | 125 | 135 | 130 | o 24 | 185 | 190 | 190 | 190 | 190 | 185 | o 24 |
| Kungsb.k. | 135 | 140 | 140 | 145 | o 24 | 185 | 185 | 185 | 185 | 185 | 175 | o 24 |
| Ylilied.k. | 45 | 45 | 35 | 35 | o 24 | — | — | 40 | 50 | 60 | 65 | e 24 |
| Lb 80 | 175 | 175 | 190 | 170 | o 24 | 220 | 220 | 250 | 245 | 245 | 225 | o 24 |
| Lb 83 | 105 | 105 | 100 | 105 | o 24 | 320 | 310 | 330 | 320 | | 320 | o 24 |
| Lb helv. 1182 | | | | | | 200 | 205 | 150 | | | | o 24 |
| Lb lact. 1183 | 90 | 165 | 195 | 200 | s 24 | 50 | 80 | | 130 | 140 | | s 24 |

Notes: e = calcium essential for growth.
s = calcium stimulates growth.
o = calcium has no effect on the growth.

IIa: strains requiring calcium ions in both media,

IIb: strains requiring calcium ions only in the medium containing citrate, and

IIc: strains not requiring calcium ions in either medium.

In addition, the first two groups can be divided into subgroups.

IIa1 and IIb1: calcium ions are essential for growth, and

IIa2 and IIb2: calcium ions stimulate the growth.

Similar groups can be formed on the basis of the experiments carried out in the basal media SII and SIIIb — these media differ from each other primarily in phosphate content (Table 11). The groups are in this case:

IIx1: the strains require calcium ions in both media,

IIx2: calcium ions stimulate the growth in SIIIb medium,

IIy2: calcium ions are not necessary for growth in medium SIIIb but stimulate the growth in medium SII, and

IIz: the strains do not require calcium ions for growth in either medium.

These results show that the importance of calcium ions is not restricted to a counteraction of the growth inhibition by citrate. It seems probable that phosphate in the concentrations employed also exerts an inhibiting effect on several strains. Calcium ions seem to counteract this inhibition as well.

The experiments proved that calcium counteracts the growth inhibition by citrate. The next step was to study possible counteracting effects of the other components of the basal media.

**Table 10.**

Grouping of strains according to the tests carried out with calcium in media SIV and SIVSS.

| IIa1 | IIa2 | IIb1 | IIb2 | IIc |
|---|---|---|---|---|
| Str 10R | Th | Str 10 | Cas J (7) | Kungsb.k. |
| Str 11R | Lb lact 1183 | Str 11 | Hatt.s. | Lb 83 |
| Str 12 | | Str 40 | | |
| Str 72 | | Str 74 | | |
| Str 77 | | Str 75 | | |
| Str 101 | | Str 100 | | |
| Ths | | Str 102 | | |
| H.th.str. | | Str 103 | | |
| | | Str 110 | | |
| | | Str 111 | | |
| | | Cas I | | |
| | | Kauhaj.k. | | |

**Table 11.**

Grouping of strains according to tests carried out with calcium in the media SII and SIIIb.

| IIx1 | IIx2 | IIy1 | IIy2 | IIz |
|---|---|---|---|---|
| Str 10R | Str 72 | Str 74 | | Str 10 |
| Str 12 | Str 102 | Str 75 | | Str 11 |
| Str 77 | Th | Str 100 | | Str 11R |
| H.th.str. | Ths | Str 101 | | Str 40 |
| | Cas I | Str 103 | | Str 110 |
| | Lb lact 1183 | Cas J (7) | | Str 111 |
| | | Ylilied.k. | | Hatt.s. |
| | | | | Kauh.k. |
| | | | | Kungsb.k. |
| | | | | Lb 83 |

**Table 12.**

Effect of calcium in the medium CIV containing 2.5 mg of sodium citrate per ml. Incubation 45 hours at 42°.

| Strain | $M\mu$mole $Ca^{++}$/ml:<br>$\mu$g $Ca^{++}$/ml: | —<br>— | 25<br>6 | 50<br>12 | 75<br>19 | 100<br>25 |
|---|---|---|---|---|---|---|
| Ths | | — | 50 | 88 | 98 | 90 |
| Str 12 | | — | — | — | — | — |
| Str 10 | | — | — | 5 | 62 | 71 |
| Th | | — | — | 13 | 13 | 87 |

*Phosphate.* In the experiments concerning calcium requirements it appeared that high phosphate concentrations inhibited the growth of several strains. Phosphate seemed to have no effect on the growth in calcium-free media when used in low concentrations, but inhibition of growth was soon observed when the concentration was increased. Calcium ions counteracted this inhibition (Tables 13, 14 and 15).

*Acetate.* As can be seen from Table 16, a much stronger growth was observed in a medium containing both calcium and acetate ions than in a medium containing acetate ions, but no calcium.

**Table 13.**

Effect of phosphate.

| Basal medium | Strain | μmole/ml | | | | | | | | Incub. hours | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 15 | 20 | 30 | 40 | 60 | 75 | 80 | | |
| CICaTa[1]) | Str 10 | — | — | | — | | — | | — | 24 | K[4]) |
| CIITa[2]) | Str 10 | 80 | | 70 | | 50 | 40 | 10 | | 24 | » |
| CII | Str 10 | 40 | | 30 | | 10 | — | — | | 24 | » |
| CIIB[3]) | Str 10 | 80 | | 80 | | 70 | 60 | 40 | | 24 | » |
| CI | Str 10 | — | | 48 | | 128 | 14 | | 19 | 48 | Na[5]) |
| » | Str 10 | 5 | | 141 | | 200 | — | | — | 48 | » |
| CIV | Str 10 | — | | — | | 6 | 6 | | 6 | 48 | » |
| » | Str 10 | 30 | | — | | — | — | | — | 48 | » |
| CI | Str 74 | — | | 4 | | 13 | 11 | | 15 | 48 | » |
| » | Str 74 | — | | 59 | | — | — | | — | 48 | » |
| CIV | Str 74 | 179 | | — | | — | — | | — | 48 | » |
| » | Str 74 | — | | — | | — | — | | — | 48 | » |
| CI | Str 75 | 0 | | 3 | | 43 | 13 | | 13 | 48 | » |
| » | Str 75 | 15 | | 109 | | — | — | | — | 48 | » |
| CIV | Str 75 | — | | — | | — | — | | 7 | 48 | » |
| CII | Str 77 | 70 | | 70 | | 40 | 10 | — | | 24 | K[4]) |
| CIIB[3]) | Str 77 | — | | — | | — | — | | — | 24 | » |
| CI | Str 101 | — | | 37 | | — | 222 | | 22 | 48 | Na[5]) |
| » | Str 101 | 9 | | 104 | | — | — | | — | 48 | » |
| CIV | Str 101 | — | | 5 | | — | — | | — | 48 | » |
| CICaTa[1]) | Hatt.s. | 10 | — | | — | | — | | | 24 | K[4]) |
| CIITa[2]) | Hatt.s. | 170 | | 230 | | 200 | 160 | 150 | | 24 | » |
| CII | Hatt.s. | 170 | | 270 | | 280 | 270 | 250 | | 24 | » |
| CIIB[3]) | Hatt.s. | 190 | | 290 | | 280 | 280 | 280 | | 24 | » |
| CI | Cas J (7) | 68 | | 134 | | 222 | 278 | | 22 | 48 | Na[5]) |
| » | Cas J (7) | 80 | | 198 | | — | — | | — | 48 | » |
| CIV | Cas J (7) | 270 | | 300 | | 300 | 7 | | 6 | 48 | » |
| » | Cas J (7) | 261 | | — | | — | — | | — | 48 | » |
| CICaTa[1]) | Lb 80 | 120 | 210 | | 190 | | 90 | — | | 24 | K[4]) |
| CIITa[2]) | Lb 80 | 150 | | 300 | | 300 | 300 | 300 | | 24 | » |
| CII | Lb 80 | 150 | | 300 | | 300 | 300 | 300 | | 24 | » |
| CIIB[3]) | Lb 80 | 150 | | 300 | | 300 | 300 | 300 | | 24 | » |

[1]) CI + 5 μg Ca++/ml + 100 μmole tartaric acid/ml.
[2]) CII + 50 μmole tartaric acid/ml.
[3]) CII + 50 μmole/ml each of malic acid, malonic acid, succinic acid, and tartaric acid.
[4]) The phosphate was added in the form of primary potassium phosphate.
[5]) The phosphate was added in the form of primary sodium phosphate.

**Table 14.**

Effect of potassium phosphate in medium CI containing acetate, glycerophosphate, succinate and tartrate (5 $\mu$mole/ml of each) tested with and without 5 $\mu$g $Ca^{++}$/ml. Incubation at 42°.

| Strain | $\mu$mole/ml | | | | | | Incubation hours |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 20 | 50 | 100 | |
| Without calcium: | | | | | | | |
| Th | 72 | 78 | 62 | 48 | 12 | — | 24 |
| Str 77 | 70 | 57 | 61 | 85 | — | — | 70 |
| Str 10 | 50 | 55 | 38 | 35 | 11 | 7 | 24 |
| Hatt.s. | 107 | 94 | 88 | 79 | 79 | 61 | 24 |
| Lb. 80 | — | 184 | 194 | 181 | 194 | 190 | 70 |
| With calcium: | | | | | | | |
| Th | 134 | 129 | 145 | 120 | 100 | 49 | 24 |
| Str 77 | 64 | 76 | 82 | 79 | 62 | 22 | 24 |
| Str 10 | 66 | 58 | 63 | 49 | 34 | 5 | 24 |
| Hatt.s. | 92 | 103 | 137 | 114 | 140 | 73 | 24 |
| Lb 80 | 175 | 181 | 195 | 199 | 220 | 198 | 70 |

*Malate, malonate, succinate, tartrate and glycerophosphate.* As very little information is available on the effect of these organic acids on the growth of lactic acid bacteria, a series of experiments were carried out with these acids. The preliminary findings are shown in Tables 17—23. These findings still require checking, but they show clearly that succinate in low concentration stimulates the growth of certain strains in the absence of calcium ions but stimulates the growth even more when calcium ions are present.

Table 24 shows results obtained in experiments where the effect of citrate was studied in a basal medium lacking acetate but containing other organic anions. Also these findings are preliminary, but it is seen that growth inhibition by citrate is stronger when organic anions other that acetate are present in the medium.

*Other organic compounds.* Table 25 shows results obtained in experiments with the following compounds: oxalacetate, $\alpha$-ketoglutarate, fumarate, formate, glycerol, lactate, pyruvate and propionate. It is not possible to draw any definite conclusions from these results, but it is interesting to note that oxalacetate inhibited the growth of *Streptococcus thermophilus* strains, and that calcium ions counteracted this inhibition to a certain extent. This particular finding has been confirmed subsequently.

The promotion of growth by calcium and other chemical compounds in the media is being investigated. The experiments carried out to date have shown that no reciprocal influence exists between calcium and certain sugars such as sucrose, glucose and lactose. It was found that some strains of *Streptococcus thermophilus* cannot utilize glucose; all the strains utilized sucrose and lactose.

**Table 15.**

Effect of calcium in media containing sodium phosphate. Incubation at 42°.

| Basal medium | Phosphate $\mu$mole/ml | Strain | $\mu$g $Ca^{++}$/ml | | | | | Incubation hours |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 5 | 10 | 20 | 40 | |
| CI | 50 | Str 10 | 78 | 173 | 141 | 149 | 142 | 42 |
| » | 50 | Str 10 | 206 | 131 | 128 | 130 | 153 | 42 |
| » | 75 | Str 10 | 260 | 288 | 241 | 162 | 216 | 42 |
| CIV | 50 | Str 10 | | — | 6 | 10 | 33 | 48 |
| » | 75 | Str 10 | — | — | — | 5 | 5 | 48 |
| CI | 50 | Str 74 | 10 | 10 | 86 | 177 | 256 | 48 |
| » | 50 | Str 74 | — | 208 | 273 | 246 | 238 | 48 |
| » | 75 | Str 74 | 315 | 220 | 220 | 250 | 280 | 48 |
| CIV | 50 | Str 74 | — | — | — | 10 | 31 | 48 |
| » | 50 | Str 74 | — | — | — | — | 11 | 48 |
| » | 75 | Str 74 | — | — | — | — | — | 48 |
| CI | 50 | Str 75 | 12 | 185 | 32 | 26 | 262 | 48 |
| » | 50 | Str 75 | 131 | 226 | 206 | 214 | 210 | 48 |
| » | 75 | Str 75 | — | 62 | 220 | 208 | 167 | 48 |
| CIV | 50 | Str 75 | 5 | — | 5 | 17 | 33 | 48 |
| » | 50 | Str 75 | — | — | — | — | 11 | 48 |
| » | 75 | Str 75 | — | — | — | — | — | 48 |
| CI | 50 | Str 101 | 60 | 134 | 136 | 133 | 134 | 48 |
| » | 50 | Str 101 | 85 | 100 | 105 | 123 | 123 | 48 |
| » | 75 | Str 101 | — | 18 | 216 | 6 | 124 | 48 |
| CIV | 50 | Str 101 | — | — | 6 | 38 | 35 | 48 |
| » | 50 | Str 101 | — | — | — | — | 9 | 48 |
| » | 75 | Str 101 | — | — | — | — | — | 48 |
| CI | 50 | Cas J (7) | 236 | 240 | 109 | 140 | 141 | 48 |
| » | 50 | Cas J (7) | 212 | 220 | 202 | 153 | 111 | 48 |
| » | 75 | Cas J (7) | 258 | 296 | 298 | 280 | 125 | 48 |
| CIV | 50 | Cas J (7) | 5 | 281 | 299 | 330 | 335 | 48 |
| » | 50 | Cas J (7) | — | — | 8 | 16 | 20 | 48 |
| » | 75 | Cas J (7) | — | — | — | — | — | 48 |

**Table 16.**

Effect of sodium acetate in medium CI containing glycerophosphate, potassium phosphate, succinate, and tartrate (5 $\mu$mole/ml of each), tested with and without calcium. Incubation at 42°.

| $\mu$g $Ca^{++}$/ml | Strain | $\mu$mole/ml | | | | | | Incubation hours |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | 50 | 100 | |
| — | Th | 31 | 79 | 94 | 97 | 101 | — | 24 |
| 5 | Th | 124 | 144 | 149 | 163 | 167 | 149 | 24 |
| — | Str 77 | — | 78 | 76 | 92 | 87 | 7 | 70 |
| 5 | Str 77 | 5 | 75 | 76 | 92 | 87 | 7 | 24 |
| — | Str 10 | 43 | 50 | 53 | 57 | 70 | 66 | 24 |
| 5 | Str 10 | 60 | 68 | 65 | 73 | 78 | 82 | 24 |
| — | Hatt.s. | 51 | 92 | 85 | 58 | 68 | 85 | 24 |
| 5 | Hatt.s. | 67 | 105 | 77 | 70 | 54 | 61 | 24 |
| — | Lb 80 | 142 | 177 | 222 | 228 | 258 | 294 | 70 |
| 5 | Lb 80 | 172 | 180 | 208 | 246 | 272 | 284 | 70 |

**Table 17.**

Effect of malic acid. Incubation 48 hours at 42°.

| Basal medium | Strain | $\mu$mole/ml | | | |
|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 |
| CICa[1]) | Th | 20 | — | — | — |
| BI | Th | 30 | 10 | — | — |
| BII | Th | 90 | — | — | — |
| CICa | Str 77 | 10 | 10 | — | — |
| BI | Str 77 | 20 | 10 | — | — |
| BII | Str 77 | — | — | — | — |
| CICa[1]) | Hatt.s. | 80 | 170 | 170 | 20 |
| BI | Hatt.s. | 70 | 10 | — | — |
| BII | Hatt.s. | 240 | 200 | — | — |
| CICa | Lb 80 | 70 | 120 | 70 | 100 |
| BI | Lb 80 | 70 | 220 | 230 | 200 |
| BII | Lb 80 | 400 | 230 | 180 | 190 |

**Table 18.**

Effect of malonic acid. Incubation 48 hours at 42°.

| Basal medium | Strain | µmole/ml | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 | 200 |
| BI | Th | 30 | — | — | — | — |
| BII | Th | 10 | — | — | — | — |
| BI | Str 77 | 20 | — | — | — | — |
| BII | Str 77 | 40 | — | — | — | — |
| BI | Str 10 | 50 | 20 | — | — | — |
| BII | Str 10 | 70 | — | — | — | — |
| BI | Hatt.s. | 170 | 30 | — | — | — |
| BII | Hatt.s. | 240 | 200 | — | — | — |
| BI | Lb 80 | 80 | 220 | 180 | 50 | 10 |
| BII | Lb 80 | 260 | 240 | 190 | — | — |

**Table 19.**

Effect of high succinic acid concentrations. Incubation 48 hours at 42°.

| Basal medium | Strain | µmole/ml | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 | 200 |
| CICa[1]) | Th | 10 | 90 | — | — | — |
| BI | Th | 40 | — | — | — | — |
| BII | Th | — | — | — | — | — |
| CICa | Str 77 | 10 | 150 | — | — | — |
| BI | Str 77 | 20 | — | — | — | — |
| BII | Str 77 | — | — | — | — | — |
| CICa | Str 10 | 10 | 120 | 50 | — | — |
| BI | Str 10 | 20 | 140 | 200 | — | 10 |
| BII | Str 10 | 60 | — | — | — | — |
| CICa | Hatt.s. | 80 | 170 | 170 | 20 | — |
| BI | Hatt.s. | 100 | 210 | 10 | — | — |
| BII | Hatt.s. | 220 | 180 | — | — | — |
| CICa | Lb 80 | 70 | 90 | 120 | 110 | 20 |
| BI | Lb 80 | 80 | 200 | 180 | 160 | 30 |
| BII | Lb 80 | 260 | 210 | 150 | 140 | — |

## Table 20.

Effect of low succinic acid concentrations in medium CI containing acetate, glycerophosphate, and potassium phosphate (5 $\mu$mole/ml of each), tested with and without $Ca^{++}$. Incubation at 42°.

| $\mu$g $Ca^{++}$/ml | Strain | $\mu$mole/ml 0 | 5 | 10 | 20 | 50 | 100 | Incubation hours |
|---|---|---|---|---|---|---|---|---|
| — | Th | 78 | 94 | 95 | 95 | — | | 24 |
| 10 | Th | 115 | 139 | 157 | 169 | 196 | — | 24 |
| — | Str 77 | 26 | 14 | — | — | — | — | 24 |
| 10 | Str 77 | 67 | 79 | 82 | 87 | 13 | — | 24 |
| — | Str 10 | 51 | 55 | 72 | 82 | 77 | — | 24 |
| 10 | Str 10 | 54 | 65 | 71 | 98 | 105 | — | 24 |
| — | Hatt.s. | 89 | 99 | 125 | 76 | 53 | | 24 |
| 10 | Hatt.s. | 95 | 88 | 108 | 65 | 61 | 20 | 24 |
| — | Lb 80 | 133 | 191 | 200 | 226 | 272 | | 70 |
| 10 | Lb 80 | 135 | 187 | 224 | 248 | 264 | 264 | 70 |

## Table 21.

Effect of tartaric acid. Incubation at 42°.

| Basal medium | Strain | $\mu$mole/ml 0 | 5 | 10 | 20 | 50 | 100 | 150 | 200 | Incubation hours |
|---|---|---|---|---|---|---|---|---|---|---|
| BI | Th | 40 | | | | — | — | — | — | 48 |
| BII | Th | — | | | | — | — | — | — | 48 |
| CICa[1]) | Th | 20 | | | | — | — | — | — | 48 |
| CIb | Th | 96 | 105 | 88 | 19 | — | — | | | 24 |
| CIbCa | Th | 118 | 132 | 141 | 142 | 79 | — | | | 24 |
| BI | Str 77 | 10 | | | | — | — | — | — | 48 |
| BII | Str 77 | — | | | | — | — | — | — | 48 |
| CICa | Str 77 | 10 | | | | 110 | — | — | — | 48 |
| CIb | Str 77 | 59 | 37 | — | — | — | — | — | | 24 |
| CIbCa | Str 77 | 98 | 101 | 100 | 78 | — | — | | | 24 |
| BI | Str 10 | 30 | | | | 10 | — | — | — | 48 |
| BII | Str 10 | 60 | | | | — | — | — | — | 48 |
| CICa | Str 10 | 10 | | | | 30 | — | — | — | 48 |
| CIb | Str 10 | 66 | 63 | 65 | 54 | — | — | — | | 24 |
| CIbCa | Str 10 | 63 | 77 | 63 | 65 | — | — | | | 24 |
| BI | Hatt.s. | 100 | | | | 210 | 10 | — | — | 48 |
| BII | Hatt.s. | 210 | | | | 190 | — | — | — | 48 |
| CICa | Hatt.s. | 10 | | | | 40 | — | — | — | 48 |
| CIb | Hatt.s. | 130 | 142 | 142 | 130 | 71 | — | — | | 24 |
| CIbCa | Hatt.s. | 131 | 126 | 117 | 123 | 93 | 25 | | | 24 |
| BI | Lb 80 | 80 | | | | 200 | 180 | 160 | 30 | 48 |
| BII | Lb 80 | 260 | | | | 200 | 170 | 130 | 10 | 48 |
| CICa | Lb 80 | 70 | | | | 120 | 120 | 130 | 10 | 48 |
| CIb | Lb 80 | 158 | 176 | 185 | 202 | 216 | — | | | 24 |
| CIbCa | Lb 80 | 159 | 183 | 206 | 218 | 234 | 185 | | | 24 |

**Table 22.**

Effect of glycero-2-phosphate in medium CI containing 5 $\mu$mole/ml each of acetate, potassium phosphate, succinate, and tartrate. Incubation at 42°.

| $\mu$g $Ca^{++}$/ml | Strain | $\mu$mole/ml | | | | | | Incubation hours |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | 50 | 100 | |
| — | Th | 34 | 87 | 97 | — | — | — | 24 |
| 10 | Th | 94 | 139 | 156 | 194 | 18 | — | 24 |
| — | Str 77 | 26 | 11 | — | — | — | — | 24 |
| 10 | Str 77 | 68 | 86 | 100 | 68 | — | — | 24 |
| — | Str 10 | 23 | 50 | 82 | 130 | 71 | — | 24 |
| 10 | Str 10 | 44 | 69 | 91 | 141 | 210 | — | 24 |
| — | Hatt.s. | 98 | 101 | 74 | 73 | 34 | — | 24 |
| 10 | Hatt.s. | 87 | 98 | 53 | 61 | 28 | — | 24 |
| — | Lb 80 | — | 178 | 214 | 218 | 280 | — | 70 |
| 10 | Lb 80 | 145 | 198 | 210 | 230 | 298 | — | 70 |

**Table 23.**

Effect of malic acid, malonic acid, succinic acid, and tartaric acid in medium CI. Incubation 48 hours at 42°.

| Strain | malic acid, malonic acid, succinic acid, tartaric acid, $\mu$mole/ml of each | | | | |
|---|---|---|---|---|---|
| | 0 | 12.5 | 25 | 37 | 50 |
| Th | — | 20 | 50 | 50 | 60 |
| Str 77 | — | 10 | 40 | 50 | — |
| Hatt.s. | 50 | 90 | 90 | 130 | 120 |
| Lb 80 | 60 | 130 | 160 | 160 | 100 |

**Table 24.**

Effect of sodium citrate in the media CI, CII, and CIV. Incubation 48 hours at 37°.

| Basal medium | Strain | $\mu$mole/ml | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 10 | 15 | 20 | 27 | 34 | 48 | 61 |
| CI | Th | 61 | 20 | 24 | — | — | — | — | — | — | — |
| CII | Th | 109 | 95 | 48 | — | — | — | — | — | — | — |
| CIV | Th | 186 | 176 | — | — | — | — | — | — | — | — |
| CI | Str 77 | — | — | — | — | — | — | — | — | — | — |
| CII | Str 77 | 126 | 95 | 80 | — | — | — | — | — | — | — |
| CIV | Str 77 | 138 | 89 | — | — | — | — | — | — | — | — |
| CI | Str 10 | — | 17 | 21 | — | — | — | — | — | — | — |
| CII | Str 10 | 67 | 59 | 68 | — | — | — | — | — | — | — |
| CIV | Str 10 | 164 | 142 | 87 | — | — | — | — | — | — | — |
| CI | Hatt.s. | 76 | 98 | 126 | 128 | 49 | 120 | — | — | — | — |
| CII | Hatt.s. | 193 | 192 | 183 | 6 | — | — | — | — | — | — |
| CIV | Hatt.s. | 272 | 180 | 127 | 7 | — | — | — | — | — | — |
| CI | Lb 80 | 65 | 88 | 133 | 141 | 164 | 138 | 129 | 26 | — | — |
| CII | Lb 80 | 176 | 142 | 139 | 122 | 43 | 4 | — | — | — | — |
| CIV | Lb 80 | 280 | 248 | 198 | 192 | 137 | — | — | — | — | — |

**Table 25.**

Effect of oxalacetate (= Oa), $\alpha$-ketoglutarate (= Kg), fumarate (= Fu), formate (= Fo), glycerol (= G), lactate (= La), pyruvate (= Py), and propionate (= Pr) in medium CI, tested with 10 $\mu$g $Ca^{++}$/ml and without calcium. Incubation 12 hours at 37°.

| Compound | Strain | without $Ca^{++}$, $\mu$mole/ml | | | | | | with $Ca^{++}$, $\mu$mole/ml | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | 50 | 100 | 0 | 5 | 10 | 20 | 50 | 100 |
| Oa | Lb 80 | 103 | — | — | — | | | 108 | 3 | — | — | | |
| Kg | Lb 80 | 79 | 117 | 130 | 167 | | | 105 | 130 | 167 | 174 | | |
| Fo | Lb 80 | 81 | 77 | 73 | 49 | 30 | 33 | 106 | 100 | 79 | 72 | 59 | 46 |
| Fu | Lb 80 | 73 | 150 | 150 | 124 | 112 | 116 | 117 | 163 | 168 | 135 | 126 | 122 |
| La | Lb 80 | 74 | 94 | 105 | 117 | 115 | 70 | 85 | 85 | 126 | 134 | 113 | 88 |
| Py | Lb 80 | 90 | 73 | 58 | 47 | 43 | 20 | 109 | 89 | 78 | 99 | 93 | 38 |
| Pr | Lb 80 | 83 | 125 | 143 | 146 | 212 | 135 | 98 | 136 | 150 | 146 | 151 | 135 |
| G | Lb 80 | 72 | 75 | 74 | 78 | 78 | 82 | 97 | 95 | 94 | 96 | 87 | 89 |
| Oa | Hatt.s. | 80 | — | — | — | | | 65 | — | — | — | | |
| Kg | Hatt.s. | 68 | 99 | 130 | 140 | | | 73 | 100 | 137 | 127 | | |
| Fo | Hatt.s. | 74 | 68 | 66 | 65 | 60 | 45 | 77 | 64 | 67 | 68 | 68 | 53 |
| Fu | Hatt.s. | 58 | 118 | 145 | 173 | 158 | 35 | 80 | 131 | 155 | 173 | 161 | 146 |
| La | Hatt.s. | 66 | 78 | 86 | 86 | 21 | — | 75 | 82 | 87 | 90 | 27 | 8 |
| Py | Hatt.s. | 71 | 69 | 69 | 58 | 46 | 36 | 69 | 72 | 63 | 62 | 52 | 37 |
| Pr | Hatt.s. | 67 | 97 | 123 | 153 | 98 | 20 | 71 | 103 | 123 | 134 | 149 | 25 |
| G | Hatt.s. | 68 | 65 | 67 | 68 | 68 | 68 | 68 | 69 | 72 | 69 | 69 | 67 |
| Oa | Str 10 | 23 | 49 | — | — | | | 31 | 65 | 97 | — | | |
| Kg | Str 10 | 32 | 33 | 41 | 68 | | | 36 | 34 | 55 | 76 | | |
| Fo | Str 10 | 28 | 22 | 17 | 19 | 15 | 9 | 36 | 30 | 19 | 20 | 12 | 9 |
| Fu | Str 10 | 14 | 19 | 21 | 36 | 24 | — | 35 | 38 | 44 | 57 | 73 | 64 |
| La | Str 10 | 29 | 22 | 16 | 13 | — | — | 34 | 32 | 24 | 20 | — | — |
| Py | Str 10 | 17 | 18 | 24 | 40 | 86 | 40 | 28 | 39 | 53 | 67 | 84 | 45 |
| Pr | Str 10 | 14 | 36 | 47 | 47 | 19 | 4 | 26 | 46 | 49 | 56 | 19 | 8 |
| G | Str 10 | 13 | 13 | 15 | 16 | 13 | 18 | 24 | 20 | 18 | 21 | 21 | 22 |
| Oa | Th | 16 | — | — | — | | | 33 | 75 | 59 | — | | |
| Kg | Th | 26 | 40 | 75 | 68 | | | 37 | 54 | 74 | 91 | | |
| Fo | Th | 21 | 31 | 29 | 25 | 14 | 7 | 37 | 47 | 40 | 28 | 18 | 14 |
| Fu | Th | 10 | 13 | 15 | 16 | 28 | — | — | — | — | — | 5 | — |
| La | Th | 24 | 19 | 22 | 15 | — | — | 43 | 49 | 29 | 4 | — | — |
| Py | Th | 15 | 15 | 78 | 33 | 97 | 117 | — | — | 4 | 3 | 6 | — |
| Pr | Th | 14 | 29 | 31 | 18 | — | — | 40 | 65 | 72 | 54 | 9 | — |
| G | Th | 11 | 10 | 9 | 10 | 12 | 12 | 26 | 26 | 25 | 28 | 28 | 28 |

## Discussion

Our investigations have mainly dealt with those lactic acid bacteria whose growth was affected by calcium ions. Very little is known about the effect of these ions on the growth of lactic acid bacteria [9], and therefore the aim of the experiments was to determine the calcium requirements of the strains studied, and to clarify the extent to which other compounds of the basal media affect these requirements and the uptake of calcium. It was found that calcium ions could not be replaced by any of the other metal ions investigated ($Mn^{++}$, $Mg^{++}$, $Fe^{++}$, $Ba^{++}$, $Sr^{++}$). The experiments also threw light upon the effects of different concentrations of metal ions on the growth of various strains. Only magnesium ions seemed to be essential to most of the strains.

The inhibition of growth by citrate can be counteracted or at least weakened by calcium. It is of interest that the required concentrations of calcium are very low compared to the citrate concentrations. On the basis of this finding and the fact that calcium also counteracts the inhibition of growth by oxalacetate one can conclude that the counteracting effect of calcium ions is not limited to citrate alone. Moreover, it is evident that in all the basal media used calcium ions have a growth promoting effect on the strains.

The fact that the growth of about 30 of the 124 strains of lactic acid bacteria investigated were affected by calcium ions must be considered a finding of great interest. As yet, however, no conclusions can be drawn about the importance of calcium ions in the metabolism of lactic acid bacteria. The results described above indicate that calcium may play a fundamental part in the metabolism of certain organic acids.

Among the 124 investigated strains of lactobacilli there were a few which require unidentified growth factors. As yet nothing can be said about the chemical nature of these growth promoting factors although they have been separated from yeast extract and tomato juice.

The role of calcium in the metabolism of the lactic acid bacteria will be investigated further and the established unidentified growth factors will be studied to determine their chemical nature.

## Summary

The nutritional requirements of 124 strains of lactic acid bacteria isolated as pure cultures from various dairy products have been studied. Some of these strains are used in cheesemaking in Finland. By growing the strains first in non-synthetic and then in synthetic media it was found that many

of them possessed very complex growth factor requirements. Several of the strains required unidentified growth factors. Particularly the *Streptococcus thermophilus* strains require calcium for growth. It is of interest that calcium ions counteract the inhibition of growth by citrate and some other organic anions. In order to make possible a further investigation of the effect of calcium, the efforts were largely concentrated on a search for new nutrient solutions with the simplest possible compositions. Such basal media, the chemical compositions of which must, of course, be known exactly, must first be found before a detailed examination of the mechanism of action of calcium can be undertaken.

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